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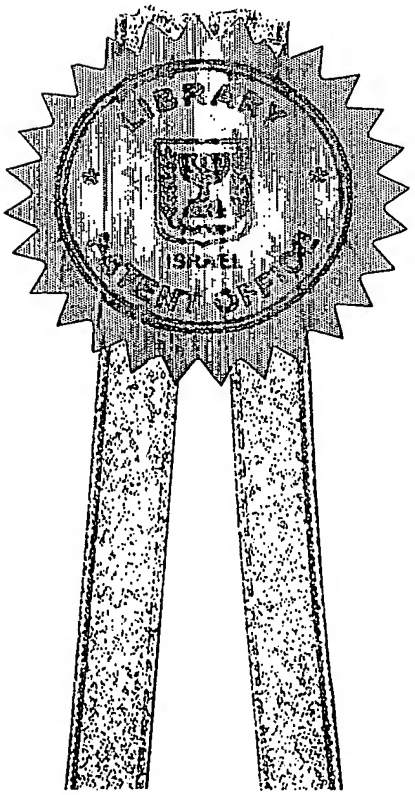

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Application for Patent

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(Name and address of applicant, and in case of body corporate-place of incorporation)

אוניברסיטת בן-גוריון בנגב
הרשות למחקר ופיתוח
ת.ד. 653
באר-שבע 84105

BEN-GURION UNIVERSITY
OF THE NEGEV
RESEARCH AND DEVELOPMENT
AUTHORITY
P.O.Box 653
Beer-Sheva 84105

Inventor: הממציא:
1. דוד מאיר צי'פמן
David M. CHIPMAN
2. זאב ברק
Ze'ev BARAK
3. סטניסלב אנג'ל
Stanislav ENGEL
4. מריה ויאזמנסקי
Maria VYAZMENSKY

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(בעברית) (Hebrew)
תהליך להכנת קטונים כיראליים ארומטיים של α – הידרוקסי על ידי שימוש באצטוהידרוקסיאציד סינטאז

(באנגלית) (English)
PROCESS FOR PREPARING CHIRAL AROMATIC α-HYDROXY KETONES USING ACETOHYDROXYACID SYNTHASE

מבקש בזאת כי ינתן לי עליה פטנט hereby apply for a patent to be granted to me in respect thereof.

*בקשת חלוקה - Application of Division	*בקשת פטנט מוסף - Application for Patent Addition	*דרישה דין קדימה Priority Claim		
*מבקשת פטנט from Application	*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
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מיום dated _____	מיום dated _____			

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P.O.A.: general / ~~individual - attached / to be filed later~~

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באר שבע 84152
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חתימת המבקש
Signature of Applicant
Luzzatto & Luzzatto
By:
Attorneys for Applicant

היום 23 בחודש ינואר שנה 2002
This 23 of ינואר of the year 2002

לשימוש הלישכה

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תהליך להכנת קטונים כיראליים ארומטיים של α – הידרוקסי על ידי שימוש באצטוהידרוקסיאציד סינטאז

PROCESS FOR PREPARING CHIRAL AROMATIC α-HYDROXY KETONES USING ACETOHYDROXYACID SYNTHASE

PROCESS FOR PREPARING CHIRAL AROMATIC α-HYDROXY KETONES USING ACETOHYDROXYACID SYNTHASE

Field of the Invention

The present invention relates to a biotransformation process for preparing chiral aromatic α-hydroxy ketones, including PAC, from optionally substituted arylaldehydes and α-ketoacids using an enzyme of the AHAS family.

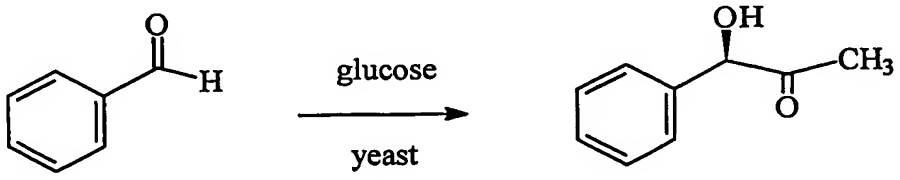
Background of the Invention

The stereospecificity (enantiospecificity) is very important for the function of bioactive compounds, such as drugs, as only one of the enantiomers usually has the desired biological activity, while the other is inactive or even toxic. Therefore, a chemical synthesis of such molecules must involve a strenuous step of separating enantiomers at some stage of the process, or said synthesis must start with a single enantiomer of a chiral precursor (a chiral synthon). The use of enzymes in the synthesis of organic compounds, beside lowering the formation of byproducts and providing high reaction rates under mild reaction conditions, obviates the above mentioned predicaments of purely chemical synthesis, since the enzymatic reactions are regioselective and stereospecific. Many biotechnologies take

advantage of biocatalysis, using either free enzymes or cells containing them.

Chiral α-hydroxyketones are versatile building blocks for the organic and pharmaceutical chemistry, e.g., for the synthesis of vitamin E, certain antifungals, antidiabetics, etc. One important chiral α-hydroxyketone is (R)-phenylacetyl carbinol (R-PAC), used as a synthon in the production of various drugs having α and β adrenergic properties, including L-ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, and phenylpropanolamine. These drugs are used as decongestants, antiasthmatics, vasoconstrictors, etc.

For many decades, (R)-PAC has been obtained by biotransformation of benzaldehyde using various species of fermenting yeast, mostly *Saccharomyces cerevisiae* (Scheme 1).



Scheme 1

The activity of the enzyme pyruvate decarboxylase (PDC) is responsible for the formation of PAC in the yeast [Hanc O. *et al.*, Naturwissenschaften 43 (1956) 498], in a synthetic side reaction accompanying the enzyme's normal decarboxylation of pyruvate to acetaldehyde.

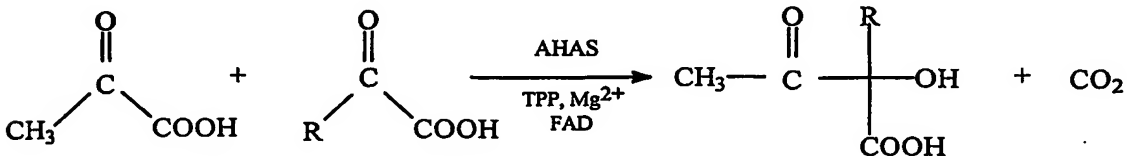
Like other biotransformations using cells, the above process is limited by toxicity of benzaldehyde towards the yeast cells, and by formation of many by-products, for example benzyl alcohol, due to the action of different cellular enzymes. These factors reduce the yield of the target product, and complicate the purification procedure. Czech patent CS 93627 (1960) describes pretreating the yeast cells by strong acids to increase their resistance toward the reaction mixture before starting the biotransformation of molasses, crude sucrose, and benzaldehyde to PAC. East German patent DD 51651 (1966) describes dosing acetaldehyde together with benzaldehyde to a yeast fermentation broth to push the reaction to the required products. WO 9004631 (1990) uses yeasts *Saccharomyces cerevisiae* or *Candida flarei* improved by mutagenesis in a biotransformation of benzaldehyde and pyruvate to PAC. The Japanese publication JP 09234090 (1997) describes the manufacture of (1R,2S)-1-phenyl-1,2-propanediol by a biotransformation of benzaldehyde and pyruvate using *Saccharomyces cerevisiae.* WO 9963103 (1999) relates to a biotransformation of a substituted aromatic aldehydes and pyruvate to the

corresponding hydroxyl ketones, comprising yeast-mediated catalysis in organic solvents. Publication JP 2000093189 (2000) describes manufacturing optical active α-hydroxyketones using genera *Torulopsis* and *Candida*. In publication WO 0144486 (2001), substituted or unsubstituted aromatic aldehydes and pyruvate condense to produce carbinol compounds using yeast, in the presence of a supercritical liquid or a liquefied gas.

Application of pure enzymes as catalysts of the desired reaction has a potential to overcome some drawbacks of the whole-cell biotransformation. The synthetic potential of pyruvate decarboxylases from *S. cerevisiae* and *Zymomonas mobilis* and benzoylformate decarboxylase from *Pseudomonas putida* has been investigated [E.g., Crout D.H.G. *et al.*, Biocatalysis 9 (1994) 1-30]. When developing a reliable industrial process based on the purified enzymes for producing (R)-PAC, two factors are of primary importance – the efficiency of (R)-PAC formation and the stability of the enzyme under production conditions. Bruhn *et al.* [Eur. J. Biochem 234 (1995) 650-6; and DE 19523269 (1996)] improved the catalytic properties of PDC of *Zymomonas mobilis* by means of site-directed mutagenesis. However, the overall efficiency of the pyruvate utilization for the carboligation reaction remained very low, with only 3.5% of the pyruvate

being converted to the desired product, and the bulk of pyruvate undergoing decarboxylation to acetaldehyde.

It is therefore an object of this invention to provide a biotransformation process for the preparation of chiral α-hydroxy ketones, including PAC, in a high yield from optionally substituted arylaldehydes and α-ketoacids. We have carried out systematic studies of acetohydroxyacid synthases (AHAS; belonging to the international classification group EC 4.1.3.18; known also as acetolactate synthase), particularly because AHAS are the target of several different classes of potent herbicides. The normal physiological reaction catalyzed by AHAS is decarboxylation-condensation of two α-keto acids (Scheme 2), producing an (S)-acetohydroxy acid, and requiring no additional driving force or redox agents. No regeneration of cofactors such as ATP or NAD are needed for the synthesis, only flavine adenine dinucleotide cofactor (FAD) and thiamin pyrophosphate (TPP) must be present.



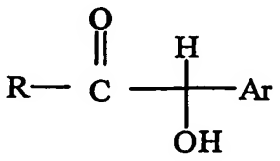
Scheme 2

We have discovered that AHAS enzymes can also utilize "unnatural" substrates [Barak Z. *et al.*, J. Bacteriol. 169 (1987) 3750-6; Gollop N. *et al.*, Biochemistry 28 (1989) 6310-7; and Ibdah M. *et al.*, Biochemistry 35 (1996) 16282-91].

It is therefore a further object of this invention to provide a biotransformation process for preparing chiral α-hydroxy ketones, including PAC, from optionally substituted arylaldehydes and α-keto acids using an enzyme of the AHAS family.

Summary of the Invention

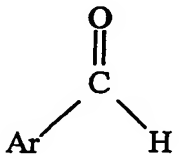
The present invention relates to a biotransformation process for preparing aromatic chiral α-hydroxy ketones, including PAC, from optionally substituted arylaldehydes and α-ketoacids in high yields using an acetohydroxyacid synthase. Said biotransformation process comprises preparing a compound of formula (I)



(I)

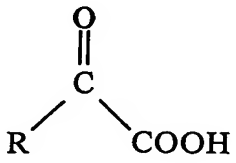
wherein R is H or C_{1-6} alkyl, and Ar is aryl, wherein said alkyl and aryl are optionally substituted by 1 to 3 substituents chosen from C_{1-3} alkyl, C_{1-3} alkoxy, F, Cl, Br, I, OH, NH_2, or NR_1R_2, wherein R_1 and R_2 can be

independently H or C_{1-4} alkyl, and said C_{1-3} alkyl can be further substituted by a substituent chosen from F, Cl, Br, I, and OH, by reacting a compound of formula (II)



(II)

with a compound of formula (III)



(III)

wherein Ar and R in formulae (II) and (III) have the meaning defined above, in the presence of a mixture comprising acetohydroxyacid synthase, a buffer, TPP, FAD, and magnesium ions.

The biotransformation process according to this invention exhibits high carboligation efficiency, since more than 99% of the compound of formula (III) reacts with the compound of formula (II), and only less than 1% is lost in decarboxylation.

Another feature of the process according to this invention is chirality of the product which is a chiral aromatic α-hydroxy ketone, predominantly (R)-arylacyl carbinol. In one aspect of the invention, the substrates are pyruvate and benzaldehyde, and the product is PAC, with enantiomeric excess of (R)-PAC exceeding 95%.

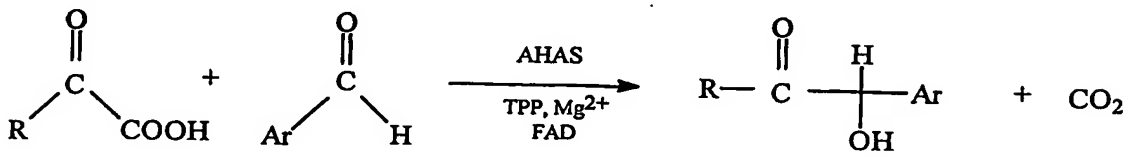
The process of this invention relates to an enzymatic reaction, taking advantage of special properties of acetohydroxyacid synthases (AHAS). The process can use plant or bacterial AHAS enzymes, which can be wild types, recombinant, engineered, and mutated, and they can be immobilized or otherwise stabilized.

Detailed Description of the Invention

It has now been found by us that AHAS enzymes can efficiently catalyze the condensation reaction of aromatic aldehydes and α-keto acids to form chiral α-hydroxyketones. We have examined the stereospecificity of the reaction, and have found that (R)-arylacetylcarbinols are formed in high enantiomeric excess.

The biotransformations were performed in a mixture containing a buffer, Mg^{2+}, TPP, FAD, two substrates, and an AHAS enzyme. The overall

balance can be depicted as shown in scheme 3, wherein R and Ar have the same meaning as defined in the Summary of the Invention.



Scheme 3

The mixture was extracted with ethyl ether at the end of the enzymatic reaction, and the products were analyzed with GC equipped with FID and MS detectors. The structures of the products were confirmed by the measurement of optical rotation, and by ^{1}H-NMR spectroscopy. The rate of consumption of benzaldehyde derivatives by AHAS was also followed by spectrophotometry .

The reaction mixture in which a biotransformation process according to the invention is performed contains a buffer keeping pH preferably from 6 to 9, its concentration being between 0.01M and 0.25 M, and which is preferably chosen from the group consisting of, but not being limited by, MES, BIS-TRIS, PIPES, BES, MOPS, TES, HEPES, TRIS, Tricine, Bicine, and phosphate. The reaction mixture further contains TPP, FAD, magnesium ions, two substrates in concentrations between 0.1 mM and 100 mM, and optionally one or more non-buffering salts in the total

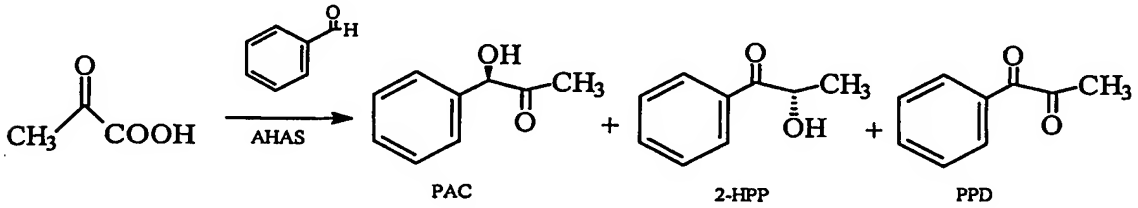
concentration from 0 to 150 mM. The concentration of enzyme is either 0.01-1.0 mg/ml or 0.1-10 U/ml, wherein the units represent μmol of carbinol formed/min. The preferred temperature of the mixture is between 25 and 40°C.

Other preferred reaction conditions for the biotransformation process according to the invention comprise pH 6.5-7.5, temperature 30°C, mild stirring, 1 mM Mg^{2+}, 0.1 mM TPP, 0.05 mM FAD, and 60 mM KCl.

It was found that the by-product formation in the reaction catalyzed by AHAS was dependent on the substrate concentrations. Further, it was found that the arylaldehyde was not consumed in any oxidative side-reaction, as indicated by an insignificant effect of the oxygen concentration on the aldehyde consumption.

In a preferred embodiment of a biotransformation according to this invention, the AHAS enzyme is the wild type AHAS isozyme II from *Escherichia coli* (WT) prepared as described in Bar-Ilan *et al.* [Biochemistry 40 (2001) 11946-54]. According to other embodiments of this invention, AHAS comprises an enzyme chosen from bacterial enzyme, yeast enzyme, fungal enzyme, and plant enzyme.

In a preferred embodiment of this invention, pyruvate reacts with optionally substituted benzaldehyde in the presence of AHAS isozyme II. We measured the concentrations of three expected compounds derived from the enzymatic condensation of pyruvate with benzaldehyde during said reaction, namely PAC, 2-hydroxypropiophenone (2-HPP) and phenylpropandione (PPD) (Scheme 4).



Scheme 4

The products 2-HPP and PPD constituted 25% of the total product when the reaction was performed at the initial substrate concentration 10 mM for both benzaldehyde and pyruvate. However, the increase of both substrates to 30 mM led to the decrease of the sum of said two undesired products to less than 1% of the total product. The natural product of the AHAS-catalyzed reaction, acetolactate (pyruvate-pyruvate condensation), was not detected. The absolute configuration of PAC was found to be R(-).

The specific activity of the AHAS enzyme used in the above biotransformation, based on the initial velocity of PAC formation, was found to be higher than1U/mg, and the efficiency of the carboligation in

the condensation of benzaldehyde and pyruvate was more than 99% based on pyruvate consumed. The enantioselectivity of the reaction was found to be ≥96%. Some advantages of the present invention can be seen from a comparison with the prior art. The following table compares biotransformations performed according to this invention and according to US 6,004,789 which used a mutant pyruvate decarboxylase from *Zymomonas mobilis*.

Table 1.

R-PAC synthesis by a mutant *Z.m.*PDC (US 6,004,789) and WT AHAS II.

	Mutant *Z.m.* PDC	WT AHAS II
Efficiency of carboligation	3.5%	>99%
Specific activity	>1 U/mg	>1 U/mg
2-HPP formation	cca. 5%	<1%
Enantioselectivity	≤95%	≥97%

The table shows that WT AHAS II exhibits better parameters in the biotransformation than a PDC enzyme, even if said PDC enzyme was used only after improving its active site by a mutation, while the AHAS enzyme was used here directly as a wild type. The active site of the AHAS

enzymes seems to be better designed to accommodate the second substrate for the condensation reaction than the active site of PDC enzymes.

In another embodiment of the present invention, a mutated WT AHAS II enzyme is used, wherein the mutation changes the specificity of the enzyme, or increases its specific activity. In one of preferred embodiments, enzyme W464L is used, which is an engineered, recombinant hexahistidine-tagged fusion protein based on WT AHAS II, overexpressed in *E.coli*, having a single mutation. Mutant W464L has lower specificity for 2-ketobutyrate than WT (specificity ratio 8 compared to 60 for WT) but the mutation increases the specific rate of the PAC production by a factor of >1.5, compared to the wild protein. This finding shows possibilities of other similar modifications of the enzyme that could change the properties of the enzyme in the desired direction, including increasing the specificity toward certain substrates, or increasing the activity.

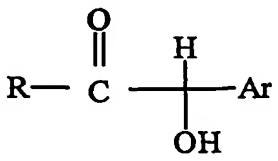
In one of embodiments of this invention, a ketoacid chosen from glyoxylic acid, 2-ketobutyric acid, and 2-ketovaleric acid reacts with an aryl that is chosen from phenyl, naphthyl, and thienyl.

In another preferred embodiment of this invention, an optionally substituted benzaldehyde reacts with pyruvate in the presence of AHAS

isozyme II. We measured the activity of WT AHAS II during the condensation of pyruvate with 3-hydroxybenzaldehyde, and found that the rate of disappearance of 3-hydroxybenzaldehyde was 87% of the rate of disappearance of unsubstituted benzaldehyde under the same conditions.

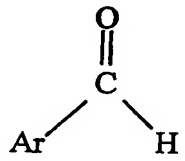
The present invention thus provides a process for the synthesis of certain chiral α-hydroxy ketones, including precursors of the drugs related to ephedrine, using an AHAS enzyme. The enzyme can be in various forms, as is known to those skilled in the art, including a crude protein or a pure enzyme, comprising either a free non-stabilized enzyme or a stabilized enzyme, eventually immobilized. The components of the enzymatic reaction can be added to the reaction mixture in one portion, or they can be added continually. The products of the biotransformation process according to this invention can be isolated according to methods known in the art, for example by extracting from the water phase to diethyl ether, followed by further purification according to known methods.

As has been said, this invention relates to a biotransformation process of preparing an aromatic α-hydroxy ketone of formula (I)



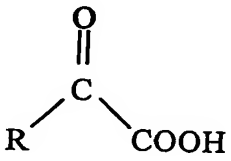
(I)

wherein R is H or C_{1-6} alkyl, and Ar is aryl, wherein said alkyl and aryl are optionally substituted by 1 to 3 substituents chosen from C_{1-3} alkyl, C_{1-3} alkoxy, F, Cl, Br, I, OH, NH_2, or NR_1R_2, wherein R_1 and R_2 can be independently H or C_{1-4} alkyl, and said C_{1-3} alkyl can be further substituted by a substituent chosen from F, Cl, Br, I, and OH, by reacting an aldehyde of formula (II)



(II)

with a ketoacid of formula (III)



(III)

wherein Ar and R in formulae (II) and (III) have the meaning defined for formula (I), in the presence of a mixture comprising acetohydroxyacid synthase, a buffer, TPP, FAD, and magnesium ions,

Examples

Materials and Procedures

Materials

Sodium pyruvate, FAD, TPP, benzaldehyde, and 3-hydroxybenzaldehyde were obtained from Sigma-Aldrich. All other materials were of analytical grade.

Biocatalyst

AHAS isozyme II from *Escherichia coli*, wild type (WT) was prepared as described in Bar-Ilan *et al.* [Biochemistry 40 (2001) 11946-54]. The mutants AHAS II W464L and AHAS II M250A were obtained as N-terminal hexahistidine-tagged proteins overexpressed in *E.coli* BL21/pRSET*ilvGM* strain. Bacterial growth, cloned genes expression and protein purification were carried out as described elsewhere (Bar-Ilan *et al.* [Biochemistry 40 (2001) 11946-54].

Enzymatic Reaction Rate

The initial rate of the enzymatic reaction was determined by the rate of benzaldehyde consumption followed spectroscopically at wavelength of 302 nm (ε = 454 $M^{-1}cm^{-1}$). Enzymatic reaction was carried out at 37° C in a 1 ml quartz cuvette in a Beckman spectrophotometer (DU 640). Reaction was initiated by the addition of AHAS II (WT or a mutant protein) to the final concentration of 0.3 mg/ml. Reaction buffer contained 0.1 M Tricine-

HCl, pH 7.5 , 1 mM $MgCl_2$, 60 mM KCl, 0.1 mM TPP, 0.05 mM FAD, and the substrates 5 mM sodium pyruvate and 5 mM benzaldehyde or substituted benzaldehyde. As much as 2% of a water miscible organic solvent such as isopropanol, dimethylsulfoxide, etc., can be added to aid in dissolution of the benzaldehyde.

The possibility that benzaldehyde is consumed in an oxidative side-reaction was tested by running the reaction under low oxygen tension (bubbling N_2 through the reaction solution); no significant effect of oxygen on the rate of benzaldehyde consumption was observed.

Biotransformation

Reaction was performed in a mixture containing 0.1 M PIPES-KOH, pH 6.5, 1 mM $MgCl_2$, 60 mM KCl, 0.1 mM TPP, and 0.05 mM FAD, in the presence of 5-30 mM sodium pyruvate and benzaldehyde at 1:1 ratio. Isopropanol (2% final) was used to aid in dissolution of benzaldehyde. AHAS isozyme II was added to the final concentration of 0.3 mg/ml to sart the reaction. At the end of the enzymatic reaction, mixture was extracted twice with 10 ml of ethyl ether. The organic phase was dried with $MgSO_4$, the solvent was removed in *vacuo*, and the products were separated and analyzed by GC-MS.

Analysis after biotransformation

The products were analyzed with GC system equipped with MS detector (Hewlett Packard). Separation was performed on a RTX-1 30m capillary column using helium as carrier gas. The injector temperature was 250° C. The column temperature regimen was: 5 min at 50° C, and then increase to 280° C at 15° C/min. The retention times were: 11.9 min for PPD, 12.7 min for PAC, and 13.1 min for 2-HPP. A flame ionization detector (300° C) was used to evaluate relative amounts of the compounds. The products identification was confirmed by ^{1}H-NMR spectroscopy. Optical rotation was measured in chloroform by a polarimeter (Perkin-Elmer).

Example 1

A 1 ml quartz cuvette containing 1 ml of the reaction mixture containing 5 mM of sodium pyruvate and 5 mM of benzaldehyde was placed in the spectrophotometer at 37° C, as described above in the paragraph Enzymatic Reaction Rate, and the reaction was started by addition of 10 µl of an enzyme solution containing 33 mg enzyme/ml. The absorbance value at 302 nm decreased linearly at a rate of 0.161 OD/min, which yielded a decrease of benzaldehyde concentration of 0.36 µmol/min, when using $\varepsilon = 454\ M^{-1}cm^{-1}$. The corresponding activity was 1.07 U/mg.

Example 2

The reaction rate was measures as described in Example 1, using as substrates 5 mM pyruvate and 5 mM 3-hydroxybenzaldehyde. The absorbance values at 346 nm decreased linearly at a rate of 0.15 OD/min, which yielded a decrease of hydroxybenzaldehyde concentration 0.3 μmol/min, when using $\varepsilon = 480\ M^{-1}cm^{-1}$. The corresponding activity was 0.93 U/mg.

Example 3

Two 20 ml flasks, each containing ten ml of the reaction mixture as described above in the paragraph Biotransformation, were shaken at 30° C. One of the flasks contained substrates at the concentrations of 10 mM, the other one of 30 mM. The reaction started by addition of 3 mg of AHAS isozyme II, and was ended after 1 h by adding 10 ml diethyl ether, and extracting, followed by second extraction with 10 ml. The extract was dried as described, evaporated to completely remove solvent and redissolved in dichloromethane. This sample was analyzed by GC-MS and GLC using FID detection. Mass spectrum showed three components corresponding to phenylacetyl carbinol (PAC), 2-hydroxypropiophenone (2-HPP), and phenylpropanedione (PPD). The following table shows the relative areas corresponding to the three products by FID.

Table 2.

GLC, FIDdetector, % areas.

Substrates	PAC	2-HPP	PPD
10 mM	80	15	5
30 mM	>99	not detectable	not detectable

Example 4

A biotransformation reaction with 30 mM substrates was performed as described in Example 3. GLC-FID showed 99% PAC. The sample was prepared as described in Example 3 and dissolved in chloroform. A solution with a concentration of 2.0 ± 0.2 g/100 mL (by GLC analysis) had an optical rotation of –0.867° as measured in a 1 cm cuvette in a polarimeter. From this value, a specific rotation $[\alpha]^{20}_{D}$ equal to 411 ± 40° can be calculated, which corresponds to 97-100% enantiomer purity.

Example 5

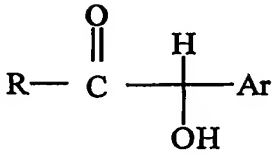
The enzymatic reaction rate was measured as described in Example 1, using the mutants of AHAS II: W464L and M250A, each comprising a single site-directed mutation. The activities 1.72 and 1.66 U/mg (1 U = 1 μmol benzaldehyde consumed/min) respectively, were found. It showed that substitution of the amino acids involved in the formation of a binding

pocket for a second substrate (according to the structural model), may lead to the improvement of the catalytic properties of the enzyme.

While this invention has been described in terms of some specific examples, many modifications and variations are possible. It is therefore understood that within the scope of the appended claims, the invention may be realized otherwise than as specifically described. It was shown that AHAS can accept different aldehydes as substrates, and it was also shown that a mutation of a wild enzyme can change the properties of the enzyme in a desired direction. A skilled person will appreciate that other acetolactate synthase or EC 4.1.3.18 enzymes can be used for realizing the present invention. The newly discovered properties of AHAS enzymes have a great potential for competing with existing biocatalytic processes, for producing a variety of chiral α-hydroxy ketones, as well as for providing new routes to other important chiral synthons.

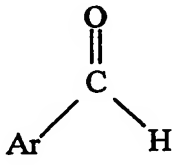
CLAIMS

1. A process of preparing an aromatic α-hydroxy ketone of formula (I)



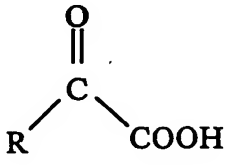
(I)

wherein R is H or C_{1-6} alkyl, and Ar is aryl, wherein said alkyl and aryl are optionally substituted by 1 to 3 substituents chosen from C_{1-3} alkyl, C_{1-3} alkoxy, F, Cl, Br, I, OH, NH_2, or NR_1R_2, wherein R_1 and R_2 can be independently H or C_{1-4} alkyl, and said C_{1-3} alkyl can be further substituted by a substituent chosen from F, Cl, Br, I, and OH; which process comprises reacting an aldehyde of formula (II)



(II)

with a ketoacid of formula (III)



(III)

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wherein Ar and R in formulae (II) and (III) have the meaning defined for formula (I); in the presence of a mixture comprising acetohydroxyacid synthase (AHAS), thiamin pyrophosphate (TPP), flavine adenine dinucleotide (FAD), magnesium ions, and a buffer.

2. A process according to claim 1, wherein one of the enantiomers of the compound of formula (I) is formed in excess.

3. A process according to claim 1, wherein the aromatic α-hydroxy ketone is chiral aromatic α-hydroxy ketone.

4. A process according to claim 1, wherein the compound of formula (I) is (R)-arylacyl carbinol.

5. A process according to claim 1, wherein the ketoacid is pyruvic acid.

6. A process according to claim 1, wherein the ketoacid is chosen from glyoxylic acid, 2-ketobutyric acid, and 2-ketovaleric acid.

7. A process according to claim 1, wherein the aryl is chosen from phenyl, naphthyl, and thienyl.

8. A process according to claim 1, wherein the aldehyde is a substituted benzaldehyde.

9. A process according to claim 1, wherein the aldehyde is benzaldehyde.

10. A process according to claim 1, wherein the compound of formula (I) is phenylacetyl carbinol (PAC).

11. A process according to claim 1, wherein the compound of formula (I) is (R)-PAC.

12. A process according to claim 10, wherein PAC constitutes more than 95% of the products of the enzymatic reaction.

13. A process according to claim 10, wherein PAC constitutes more than 99% of the products of the enzymatic reaction.

14. A process according to claim 11, wherein (R)-PAC constitutes more than 90% of PAC produced in the enzymatic reaction.

15. A process according to claim 11, wherein (R)-PAC constitutes more than 95% of PAC produced in the enzymatic reaction.

16. A process according to any one of claims 1 to 15, wherein AHAS comprises an enzyme of bacterial origin.

17. A process according to any one of claims 1 to 15, wherein AHAS comprises an enzyme chosen from yeast enzyme, fungal enzyme, and plant enzyme.

18. A process according to any one of claims 1 to 15, wherein AHAS comprises a wild type protein.

19. A process according to any one of claims 1 to 15, wherein AHAS comprises a recombinant protein.

20. A process according to any one of claims 1 to 15, wherein AHAS comprises an engineered protein.

21. A process according to any one of claims 1 to 15, wherein AHAS comprises a mutant protein.

22. A process according to any one of claims 1 to 15, wherein AHAS comprises isozyme II protein from *Escherichia coli*.

23. A process according to any one of claims 1 to 15, wherein AHAS comprises a histidine-tagged protein.

24. A process according to any one of claims 1 to 15, wherein AHAS comprises specific directed mutants of AHAS II overexpressed in host cells.

25. A process according to any one of claims 1 to 24, wherein AHAS comprises a stabilized enzyme.

26. A process according to any one of claims 1 to 25, wherein AHAS comprises an immobilized enzyme.

27. A biotransformation process according to any one of claims 1 to 26, wherein all the components of the enzymatic reaction are added to the reaction mixture in one portion.

28. A biotransformation process according to any one of claims 1 to 26, wherein some of the components of the enzymatic reaction are added to the reaction mixture in more portions or continually.

29. A process according to claims 27 or 28, wherein pH of the mixture is from 6 to 9.

30. A process according to claims 27 or 28, wherein pH of the mixture is from 6.5 to 7.5.

31. A process according to claims 27 or 28, wherein the mixture comprises a buffer chosen from the group consisting of MES, BIS-TRIS, PIPES, BES, MOPS, TES, HEPES, TRIS, Tricine, Bicine, and phosphate.

32. A process according to claims 27 or 28, wherein the buffer has a concentration between 0.01 M and 0.25 M.

33. A process according to claims 27 or 28, wherein the aldehyde and the ketoacid are added to concentrations between 2 mM and 100 mM.

34. A process according to claims 27 or 28, wherein TPP and FAD are added to concentrations between 0.02 mM and 0.2 mM.

35. A process according to claims 27 or 28, wherein magnesium ions are added to a concentration between 0.2 mM and 2 mM.

36. A process according to claims 27 or 28, wherein an AHAS enzyme is added to a concentration between 0.01 mg/ml and 1.0 mg/ml.

37. A process according to claims 27 or 28, wherein an AHAS enzyme is added to a concentration between 0.1 and 10 U/ml.

38. A process according to claims 27 or 28, wherein the temperature of the mixture is between 25 and 40°C.

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